THE JOURNAL OF BIOLOGICAL CHEMISTRY
Vol. 274, No. 43, Issue of October 22, pp. 30540–30549, 1999

Printed in U.S.A.

Inactivation of the Open Reading Frame *slr*0399 in *Synechocystis* sp. PCC 6803 Functionally Complements Mutations near the Q_A Niche of Photosystem II

A POSSIBLE ROLE OF Slr0399 AS A CHAPERONE FOR QUINONE BINDING*

(Received for publication, May 24, 1999, and in revised form, August 5, 1999)

Svetlana Ermakova-Gerdes‡ and Wim Vermaas

From the Department of Plant Biology and Center for the Study of Early Events in Photosynthesis, Arizona State University, Tempe, Arizona 85287-1601

The *Synechocystis* sp. PCC 6803 triple mutant D2R8 with V247M/A249T/M329I mutations in the D2 subunit of the photosystem II is impaired in Q_A function, has an apparently mobile Q_A, and is unable to grow photoautotrophically. Several photoautotrophic pseudorevertants of this mutant have been isolated, each of which retained the original *psb*DI mutations of D2R8. Using a newly developed mapping technique, the site of the secondary mutations has been located in the open reading frame *slr*0399. Two different nucleotide substitutions and a deletion of about 60% of *slr*0399 were each shown to restore photoautotrophy in different pseudorevertants of the mutant D2R8, suggesting that inactivation of Slr0399 leads to photoautotrophic growth in D2R8. Indeed, a targeted deletion of *slr*0399 restores photoautotrophy in D2R8 and in other *psb*DI mutants impaired in Q_A function. Slr0399 is similar to the hypothetical protein Ycf39, which is encoded in the cyanelle genome of *Cyanophora paradoxa*; in the chloroplast genomes of diatoms, dinoflagellates, and red algae; and in the nuclear genome of *Arabidopsis thaliana*. Slr0399 and Ycf39 have a NAD(P)H binding motif near their N terminus and have some similarity to isoflavone reductase-like proteins and to a subunit of the eukaryotic NADH dehydrogenase complex I. Deletion of *slr*0399 in wild type *Synechocystis* sp. PCC 6803 has no significant phenotypic effects other than a decrease in thermotolerance under both photoautotrophic and photomixotrophic conditions. We suggest that Slr0399 is a chaperone-like protein that aids in, but is not essential for, quinone insertion and protein folding around Q_A in photosystem II. Moreover, as the effects of Slr0399 are not limited to photosystem II, this protein may also be involved in assembly of quinones in other photosynthetic and respiratory complexes.

The cyanobacterium *Synechocystis* sp. PCC 6803 is a useful molecular genetic system to study oxygenic photosynthesis and cell physiology of photosynthetic microorganisms. The organism is unique in that it combines several desirable properties: (i) *Synechocystis* sp. PCC 6803 is spontaneously transformable and incorporates exogenous DNA into its genome via double-homologous recombination (1, 2); (ii) the strain can grow (photo)heterotropically, thus enabling the creation of *Synechocystis* sp. PCC 6803 strains impaired in photosystem I (PS I)[1] and/or photosystem II (PS II) function (for a recent review, see Ref. 3); and (iii) the entire genome sequence of *Synechocystis* sp. PCC 6803 is known (4). Because of these properties, a variety of molecular genetic approaches have been applied to study the role of particular proteins in this organism.

One of these approaches is the mapping and characterization of pseudorevertants, which carry second-site mutations restoring viability under conditions that are lethal for the original mutants. In the case of mutants that are impaired in photosynthesis, pseudorevertants with improved photosynthetic function and with secondary mutations in genes coding for proteins with known function have been isolated and analyzed (for example, see Refs. 5–11). These genes may be identical to the ones carrying the original mutations or may be at a different locus.

In eukaryotic genetic systems such as *Chlamydomonas reinhardtii* or *Arabidopsis thaliana* mapping of a site of a suppressor mutation in an unrelated gene is a time-consuming and complicated project. However, with the advent of a known genomic sequence, in *Synechocystis* sp. PCC 6803 the process of mapping of genes that contain second-site mutations has been simplified and accelerated by development of a novel technique of functional complementation with size-separated restriction fragment pools (3). In this way, a functionally complementing gene can be identified using the pseudorevertant DNA without the need for library construction.

In the present study we have applied this technique to characterize frequently occurring photoautotrophic pseudorevertants of the obligate photoheterotrophic D2R8 mutant (12), which has primary mutations in the Q_A-binding niche of the D2 protein that is part of the PS II reaction center complex. Surprisingly, the location of these pseudoreversions was found to map to *slr*0399, an open reading frame coding for a protein of an unknown function. As will be presented in this paper, we suggest that Slr0399 may function as a chaperone helping to insert Q_A into its site. Even though chaperone function has been well established for folding of soluble proteins (reviewed

* This work was supported by National Science Foundation Grant MCB 9728400 (to W. V.). The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

‡ To whom correspondence should be addressed: Dept. of Plant Biology and Center for the Study of Early Events in Photosynthesis, Arizona State University, Box 871601, Tempe, AZ 85287-1601. Tel.: 480-965-3698; Fax: 480-965-6899.

[1] The abbreviations used are: PS I, photosystem I; PS II, photosystem II; PCR, polymerase chain reaction; Q_A, the primary electron-accepting plastoquinone in PS II; Q_B, the second electron-accepting plastoquinone in PS II; DCMU, 3-(3,4-dichlorophenyl)-1,1-dimethylurea; DQ, tetramethyl-*p*-benzoquinone (duroquinone); PQ, plastoquinone; vitamin K_1, phylloquinone; kb, kilobase pair(s); HPLC, high performance liquid chromatography; IRL, isoflavone reductase-like protein; TES, *N*-tris (hydroxymethyl)methyl-2-aminoethanesulfonic acid.

recently in Refs. 13 and 14), much less is known about the possible involvement of chaperones in folding of membrane protein complexes and in insertion of cofactors.

EXPERIMENTAL PROCEDURES

Growth Conditions—Synechocystis sp. PCC 6803 cells were grown at 30 °C in BG-11 medium (15) supplemented with 5 mM glucose at the light intensity of 50 μmol photons m^{-2} s^{-1}. Liquid medium was perfused with sterile air. Solid medium was supplemented with 1.5% agar, 0.3% sodium thiosulfate, and 10 mM TES/NaOH buffer, pH 8.2. The PS II inhibitor atrazine (20 μM) was added to solid medium for maintenance of obligate photoheterotrophic mutants with defects in PS II in order to avoid inadvertent selection for photoautotrophic (pseudo)revertants.

Chromosomal DNA Isolation and Fractionation—For the isolation of genomic DNA, *Synechocystis* sp. PCC 6803 cells were pelleted and incubated at 37 °C for 20 min with 2 ml of saturated NaI solution/g (wet weight) of cells. After dilution of NaI with 5–10 volumes of water, cells were pelleted by centrifugation and resuspended in 50 mM Tris-HCl, pH 8.0, 50 mM NaCl, and 5 mM EDTA; lysozyme was added to a final concentration of 7 mg/ml. After incubation at 37 °C for 20 min, *N*-lauryl sarcosine was added to 1% (w/v) final concentration, and cells were incubated at 37 °C for 20 min to induce cell lysis. DNA was extracted several times with phenol, and then with a 1:1 phenol:chloroform mix. During extraction, very gentle agitation was used to avoid extensive fragmentation. After precipitation with ethanol, DNA was resuspended in TE buffer and ammonium acetate was added to a final concentration of 2.5 M. The solution was incubated on ice for 1 h and cleared by centrifugation in a microcentrifuge at 4 °C. Ethanol (1.5 volumes) was added to the supernatant to precipitate the DNA. DNA was pelleted by centrifugation, washed in 70% ethanol, and resuspended in TE (10 mM Tris-HCl, pH 7.6, and 2 mM EDTA) buffer. After digestion of the chromosomal DNA with restriction endonucleases and size fractionation on a 0.4% agarose gel in TAE buffer (40 mM Tris-acetic acid, pH 8.0, and 1 mM EDTA), the gel was soaked in distilled water for 30 min and then in BG-11 with gentle agitation, stained with ethidium bromide, and each lane of the gel was sliced into 20–25 pieces, each representing a specific size range. DNA fragments from each size range were eluted from the agarose as follows. Agarose slices were incubated at −80 °C overnight, thawed at 37 °C for 30–60 min, spun in a microcentrifuge at 4,000 rpm for 5 min, and then at 14,000 rpm for another 10 min. The supernatant was used for *Synechocystis* sp. PCC 6803 transformation without further purification.

Complementation of the D2R8 Mutant—D2R8 mutant cells were grown to mid-log phase (OD_{730} about 0.5 as measured on a Shimadzu UV-160 spectrophotometer), were concentrated to OD_{730} = 10, and 1 ml of this culture was spread on a BG-11 agar plate. After the spread suspension had dried on the plate, 50–100 μl of each DNA sample (10–15 different samples per plate, each at a different spot) were applied directly on the cell lawn and allowed to dry. Photoautotrophic transformants were visible after 8–10 days of incubation at 30 °C in the light at 50 μmol photons m^{-2} s^{-1}.

Oxygen Evolution Assay—The steady-state rate of oxygen evolution was determined in intact cells on a Gilson model KM oxygraph at a chlorophyll concentration of 7 μg/ml. Measurements were performed in BG-11 medium buffered with 25 mM HEPES/NaOH, pH 7.0, with or without electron acceptors (0.1 mM 2,5-dimethyl-*p*-benzoquinone (DMBQ) and 0.5 mM $K_3[Fe(CN)_6]$). The light from a 150-watt xenon arc lamp was filtered through water and through a Schott OG-570 filter and was saturating for maximal electron transfer rates (1800 μmol photons m^{-2} s^{-1}).

PS II Quantitation—PS II quantitation in whole cells on a chlorophyll basis using atrazine-replaceable [^{14}C]diuron binding was performed as described in Ref. 16.

PS II Fluorescence Induction Measurements—Chlorophyll *a* fluorescence induction and decay of the variable fluorescence were measured in intact cells on a PAM fluorometer (Walz, Germany) as described in Ref. 17.

Quinone Extraction and Fractionation—One liter of *Synechocystis* sp. PCC 6803 culture was harvested in mid-exponential growth phase (OD_{730} between 0.4 and 0.6), and thylakoid membranes were isolated as described in Ref. 18. The quinone pool in isolated thylakoids was oxidized by incubation with 0.5 mM $K_3[Fe(CN)_6]$ in the dark for 10 min. Quinones and other prenyllipids were extracted by incubation with chloroform/methanol/water (1:1:0.3) for 1 h at 37 °C in the dark under nitrogen with agitation according to Ref. 19. The ratio of chloroform/methanol/water in the extract was subsequently adjusted to 3:2:1, and the tubes were centrifuged at 5,000 × *g* for 5 min to separate the phases. The lower, dark green phase was evaporated in the dark under nitrogen, and pigments were dissolved in 500 μl of a chloroform/methanol mix (2:1). HPLC analysis was performed on a Beckman solvent pump model 126 HPLC instrument fitted with a Phenomenex Prodigy, ODS, 5-μm reverse phase column (250 × 4.6 mm). Per HPLC run, 100 μl of prenyllipids isolate in the chloroform/methanol mix was injected onto the column. A combination of linear gradients from the initial methanol/water (9:1) mixture to methanol/isopropanol/hexane (2:1:1) was run for 30 min, followed by a 8-min elution with methanol/isopropanol/hexane mixture, at a flow rate of 1.5 ml/min (20). The absorbance of the eluate was monitored at 263 nm on a Beckman model 166 absorbance detector. The data were processed with the System Gold software (Beckman). Vitamin K_1 (Aldrich) was used as a standard for quantification of the amount of quinone detected. Quinone peaks were eluted and spectrally analyzed in oxidized form and after reduction with $NaBH_4$. Peak assignments were made on the basis of comparison with published absorption spectra (21, 22) of the oxidized and reduced quinone compounds.

RESULTS

Isolation and Characterization of Pseudorevertants of the psbDI Triple Mutant D2R8—The *Synechocystis* sp. PCC 6803 mutant D2R8 does not grow photoautotrophically due to two mutations in the Q_A-binding de-loop of the D2 protein (V247M and A249T) that greatly alter the properties of Q_A and cause Q_A to be apparently mobile and replaceable by other quinones (12). This mutant lacks *psb*DII (the second gene coding for the D2 protein), and has three mutations in *psb*DI, leading to a V247M/A249T/M329I mutation combination. When the obligate photoheterotrophic D2R8 mutant was plated in the absence of glucose, photoautotrophic colonies were found to appear frequently. The estimated frequency was 10^{-5} to 10^{-6}, which was about 2 or 3 orders of magnitude higher than the frequency of true reversion that we observe for obligate photoheterotrophic single-base change mutants.

In order to determine the genetic cause of the photoautotrophic nature of the D2R8 derivatives, the *psb*DI gene from three independent photoautotrophic D2R8 derivatives (D2R8R1, D2R8R2, and D2R8R3) was amplified by PCR and sequenced. Interestingly, in all three strains, the mutations V247M, A249T, and M329I remained present in the *psb*DI gene. Therefore, the three photoautotrophic strains were clearly pseudorevertants. Moreover, no additional mutations were found in *psb*DI that was isolated from the revertants. Corroborating these findings, PCR-amplified *psb*DI from the pseudorevertants failed to transform the initial D2R8 mutant to photoautotrophy, indicating that the site of pseudoreversion in the three pseudorevertant strains was located outside of *psb*DI. Total genomic DNA isolated from the same pseudorevertants transformed the D2R8 mutant to photoautotrophy with high frequency, establishing that the pseudoreversion of D2R8 to photoautotrophic growth is due to a single genetic event. We find cotransformation of different loci to be uncommon in *Synechocystis* sp. PCC 6803.

Mapping of the Site of Pseudoreversion—Several genes or gene clusters coding for structural PS II proteins that might have domains close to the Q_A site (*psb*A2, *psb*A3, *psb*C, *psb*H, and *psb*EFLJ) were amplified by PCR from genomic DNA of the three pseudorevertants. These PCR products were examined for their ability to transform the original mutant to photoautotrophy. All of these genes tested failed to complement D2R8, indicating that they did not contain the site of the secondary mutation.

In order to identify the locus or loci responsible for the pseudoreversion in the three strains, a novel mapping technique of functional complementation with size-separated restriction fragment pools was applied (3), making use of the genomic restriction map of *Synechocystis* sp. PCC 6803 that has been constructed for 16 enzymes based on the genomic

TABLE I
Mapping of the secondary mutation in D2R8R1 by functional complementation using size-separated restriction fragment pools

Restriction enzyme	As determined from complementation test	As determined from comparison with the *Synechocystis* sp. PCC 6803 restriction map	
	Size range of complementing DNA fraction	Location of the complementing fragment within the genome	Size of the complementing fragment
	kb		*kb*
*Bgl*II	23–35	2,131,568–2,160,099	28.53
*Eco*RI	4–4.5	2,147,054–2,151,425	4.37
*Eco*RV	16–18	2,138,546–2,156,352	17.81
*Pst*I	9–11	2,140,213–2,150,458	10.25
*Sca*I	19–23	2,127,089–2,149,574	22.49
*Sma*I	17–19	2,144,053–2,163,009	18.96
*Xba*I	11–14	2,140,080–2,151,209	11.13
*Bam*HI[a]	None	2,146,114–2,149,444	3.33
*Kpn*I[a]	None	2,146,190–2,189,236	43.05
*Nhe*I[a]	None	2,146,624–2,152,677	6.05

[a] None of the DNA fractions of D2R8R1 generated by these three enzymes could complement D2R8 to photoautotrophic growth. The sizes and genome locations of the corresponding restriction fragments harboring the secondary mutation were determined after the locus of pseudoreversion had been mapped based on complementation test results for the other restriction enzymes.

sequence. The genomic DNA from pseudorevertant D2R8R1 was isolated and purified gently to avoid fragmentation. About 25 μg of genomic DNA was completely digested with one of the following 10 enzymes (*Bam*HI, *Bgl*II, *Eco*RI, *Eco*RV, *Kpn*I, *Nhe*I, *Pst*I, *Sca*I, *Sma*I, and *Xba*I) and size-separated on a 0.4% agarose gel. Each of the 10 lanes of the gel was cut into 20–25 fractions, containing DNA fragments of size categories between 1 and 35 kb. Every fraction was collected in a separate microcentrifuge tube, and DNA was extracted and used to transform the original D2R8 mutant as described under "Experimental Procedures." The capability to perform photoautotrophic growth was used as the selection criterion. Only one DNA fraction in each of seven restriction digestions complemented the D2R8 mutant, indicating that these fractions contained the restriction fragment carrying the secondary mutation (Table I). However, no complementation was observed after transformation with DNA fractions generated by restriction with *Bam*HI, *Kpn*I, and *Nhe*I (Table I). This may indicate that either (i) a restriction site of these enzymes is too close to the locus of the secondary mutation, not leaving a large enough flanking region to facilitate efficient homologous recombination in *Synechocystis* sp. PCC 6803; or (ii) the restriction fragment containing the secondary mutation generated by these enzymes was less than 1 kb or longer than 35 kb and was not represented in the complementation test.

The size ranges of the seven restriction fragments that led to functional complementation of the original mutant were compared with the size-sorted restriction map of the entire *Synechocystis* sp. PCC 6803 chromosome (3) in order to determine a single region in the genome that fitted this unique restriction pattern. Only one genomic location was found to yield restriction fragments compatible with the sizes observed for each of the seven complementing restriction fragment collections. This location was 2,147,054–2,149,574 base pairs (numbering according to CyanoBase Kazusa DNA Research Institute, Japan), corresponding to a 2,528-base pair *Eco*RI/*Sca*I fragment. This region of the genome should contain the site of the pseudoreversion.

To verify this finding, this *Eco*RI/*Sca*I fragment was amplified by PCR from pseudorevertants D2R8R1, D2R8R2, and D2R8R3, and from the wild type, cloned into the pACYC184 vector (yielding plasmids pD2R8R1, pD2R8R2, pD2R8R3, and pWT, respectively), and used to transform the D2R8 mutant. Indeed, PCR products cloned from the pseudorevertants, but not the wild type, could transform the original mutant to photoautotrophy.

The Genome Region Containing the Site of Pseudoreversion—As shown in Fig. 1*A*, the complementing 2,528-base pair *Eco*RI/*Sca*I fragment contained two complete open reading frames (*slr*0398 and *slr*0399) and two partial ones (*slr*0397 and *slr*0400) (Ref. 4; CyanoBase). Slr0397 and Slr0398 have no significant similarity to other open reading frames in the data base, whereas Slr0399 is similar to the polypeptide predicted to be encoded by an open reading frame (*ycf*39) found in chloroplasts of non-green algae. Slr0400 is similar to the putative protein encoded by *yfj*B in *Escherichia coli.* In order to determine which of these open reading frames contained the site of the pseudoreversions, the inserts in plasmids pD2R8R2 and pD2R8R3 were digested, size-separated on an agarose gel, purified, and used in a complementation test (Fig. 1*B*). In both cases the smallest complementing region was determined to be a 1.16-kb *Bst*EII-*Spe*I fragment containing *slr*0399 only (Fig. 1*B*). Sequencing of *slr*0399 from pseudorevertants D2R8R1, D2R8R2, and D2R8R3 showed that D2R8R1 and D2R8R3 contained point mutations within this open reading frame, leading to amino acid substitutions Y291C and R254H, respectively. Surprisingly, D2R8R2 carried a large deletion between the codons for Thr-128 and Leu-255, which also introduced a frameshift. This led to the loss of the C-terminal 60% of Slr0399 in D2R8R2. These results suggest that inactivation of *slr*0399 in the D2R8 *psb*DI mutant leads to restoration of PS II function in this mutant.

Complementation of PS II Mutants with slr0399 from Pseudorevertants—An important question to ask was whether inactivation of *slr*0399 could restore PS II function in various PS II mutants, or whether this effect was limited to the mutant D2R8 only. For this purpose, the plasmids pD2R8R2, pD2R8R3, and pWT were used to transform different obligate photoheterotrophic *Synechocystis* sp. PCC 6803 strains with mutations in *psb*DI, *psb*A2, or *psb*B. The results of this complementation test are presented in Table II and demonstrate that several different mutations in the Q_A-binding niche of D2 (V247M/A249T, S254F, and G258D) can be complemented to photoautotrophic growth by inactivation of *slr*0399 in *Synechocystis* sp. PCC 6803. However, the absence of complementation in the mutant A260V, which has no functional PS II centers and no oxygen evolution, suggests that photoautotrophic growth can be restored by a secondary mutation in *slr*0399 only in mutants with some PS II activity still present.

Functional complementation with pseudorevertant DNA was not observed in any of the strains where the introduced PS II mutation affected regions other than the Q_A site. This strongly suggests that in PS II Slr0399 affects solely the Q_A-binding niche. However, strains carrying mutations a few residues away from the changes in D2R8 could also be complemented by mutated Slr0399, indicating that Slr0399 may

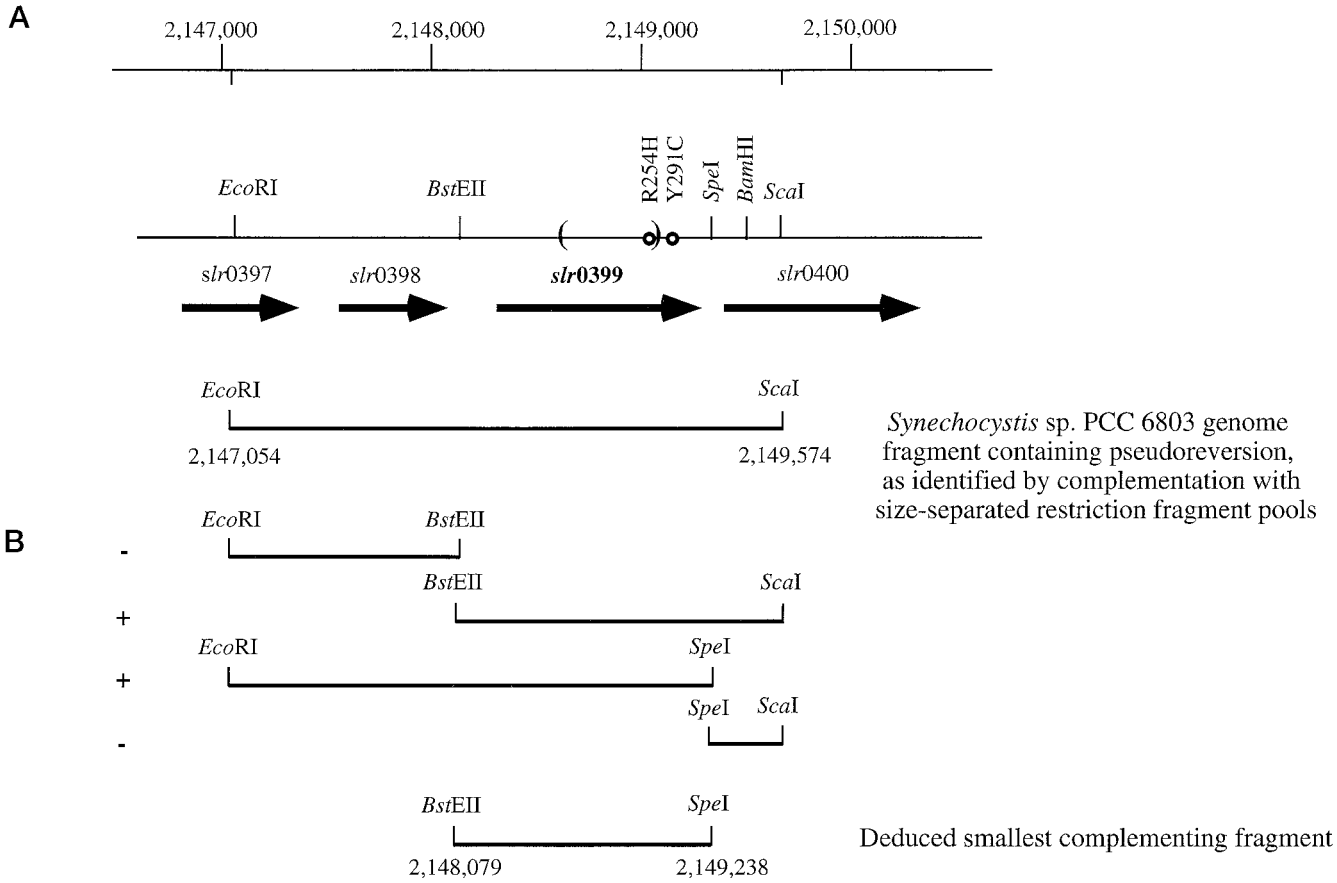

FIG. 1. **Localization of the second-site mutation in photoautotrophic D2R8 pseudorevertants.** *A*, map of the *Synechocystis* sp. PCC 6803 chromosome region containing the locus of the secondary mutations. Numbering of nucleotides and open reading frames is according to CyanoBase. The sites of the secondary mutations in the pseudorevertants D2R8R1 and D2R8R3 (residues Tyr-291 of Slr0399 changed to Cys and Arg-254 to His) are marked by *circles*, and the deletion in D2R8R2 is indicated by *parentheses*. *B*, localization of the secondary mutation sites within the *Eco*RI/*Sca*I genome fragment that was identified by complementation with the size-separated restriction fragment pools. Plasmid fragments generated from cloned *Eco*RI/*Sca*I regions of the pseudorevertants were used for transformation of the D2R8 mutant. Restriction enzymes used to generate these fragments have been indicated. The ability of the fragments to complement has been indicated by + in front of the fragments. A − indicates the lack of functional complementation.

interact with the Q_A site as a whole rather than with individual residues.

Inactivation of slr0399—To verify the notion that deletion of a large part of Slr0399 leads to photoautotrophic growth in previously obligate photoheterotrophic PS II mutants with changes at the Q_A site, a plasmid was constructed (pΔslr0399) in which a 620-base pair *Bsa*BI/*Spe*I fragment near the 3′ end of *slr*0399 was replaced by the kanamycin (Km) resistance cassette from pUC4K. To rule out possible polar effects of this deletion and/or Km insertion on the transcription of sequences located downstream of *slr*0399, another DNA construct was designed that contained intact *slr*0399, but where the Km resistance cassette was inserted at the *Spe*I restriction site adjacent to the stop codon of *slr*0399. Both constructs were used to transform the *Synechocystis* sp. PCC 6803 wild type, the D2R8 strain, and the D2 mutant S254F (see Table II), selecting for resistance to kanamycin. The complete segregation of the introduced deletions was demonstrated by PCR (Fig. 2); as *Synechocystis* sp. PCC 6803 contains multiple genome copies per cell, demonstration of segregation prior to functional analysis is essential.

As expected, targeted inactivation of *slr*0399 restored photoautotrophic growth in the photoheterotrophic *psb*DI mutants D2R8 and S254F (Table III). However, *slr*0399 inactivation had essentially no effect on the properties of the wild type (Table III). The photoautotrophic growth rate, the PS II content (as determined from the ratio of chlorophyll and the number of DCMU binding sites), the affinity of PS II for radiolabeled DCMU, the chlorophyll *a* content per cell, and the relative amount of variable fluorescence remained unchanged in wild type upon *slr*0399 inactivation. Moreover, the 77K chlorophyll fluorescence emission characteristics as well as the rate of photoinactivation of PS II electron transport upon illumination with saturating light remained unchanged (data not shown).

However, PS II properties of the D2R8 and S254F mutants were altered significantly upon introduction of the *slr*0399 inactivation construct, becoming more like those of the wild type. The photoautotrophic doubling time of the deletion mutants was 16–21 h, somewhat slower than that of the wild type, and the amount of PS II was increased 3-fold in both D2 mutants to about half of that in the wild type. Upon introduction of the *slr*0399 inactivation construct the dissociation constant of DCMU decreased from 66 and 31 nM in the initial mutants D2R8 and S254F, respectively, to values comparable to those in the wild type (Table III).

Insertion of a Km resistance cassette immediately downstream of *slr*0399 had no measurable phenotypic effect in D2R8, S254F, or the wild type (data not shown). This confirms that the phenotypic effects observed in the D2R8 pseudorevertants and in the D2 mutant strains with inactivated *slr*0399 are in fact due to *slr*0399 inactivation rather than to effects on expression levels of genes that happen to be cotranscribed with *slr*0399.

Slr0399 Effects on the D2 Mutants D2R8 and S254F—The

TABLE II

Functional complementation of different obligate photoheterotrophic PS II mutants by transformation with plasmids pD2R8R1 and pD2R8R2, which contain the mutant slr0399 alleles from two D2R8 pseudorevertants, and with plasmid pWT containing wild type slr0399

The ability of the plasmids to complement has been indicated by +, and the lack of functional complementation has been indicated by −.

Mutant	Location of mutation(s)	Oxygen evolution in Hill reaction	Complementation to photoautotrophic growth by *slr*0399 from:		
			Wild type	D2R8R1 (Y291C)	D2R8R2 (Δ128–255)
*psb*DI mutants (D2 protein)					
S79F[a]	AB-loop, in lumen	+	−	−	−
V247M/A249T[b]	Q_A niche	+	−	+	+
S254F[c]	Q_A niche	+	−	+	+
G258D[c]	Q_A niche	+	−	+	+
A260V[c]	Q_A niche	−	−	−	−
G278D[c]	E-helix	+	−	−	−
G285S/G288D[c]	E-helix	−	−	−	−
R294W[c]	C terminus, in lumen	+	−	−	−
*psb*A2 mutant (D1 protein)[d]					
Δ(N266–N267)	Q_B niche	−	−	−	−
*psb*B mutants (CP47 protein)[e]					
Δ(T271–K277)	E-loop, in lumen	−	−	−	−
Δ(K277–E283)	E-loop, in lumen	+	−	−	−
Δ(T304–L309)	E-loop, in lumen	−	−	−	−
Δ(P422–E428)	E-loop, in lumen	+	−	−	−

[a] Described in Ref. 23.
[b] These are the mutations present in the D2R8 strain (12).
[c] S. Ermakova-Gerdes and W. Vermaas, unpublished data.
[d] Described in Ref. 24.
[e] Described in Ref. 25.

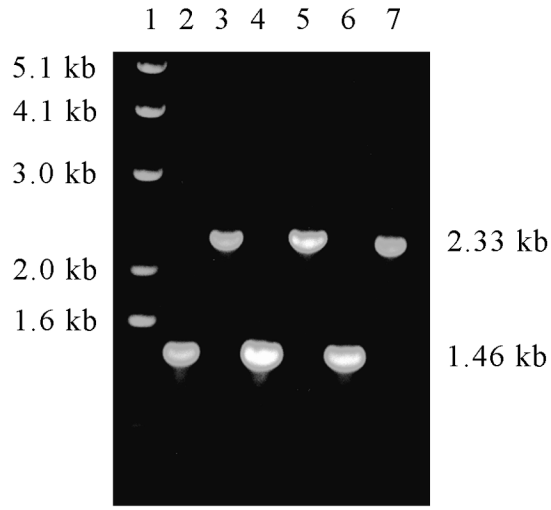

FIG. 2. **PCR amplification of the *slr*0399 gene using as templates total chromosomal DNA from the wild type (*lane 2*), *slr*0399$^-$ (*lane 3*), D2R8 (*lane 4*), D2R8/*slr*0399$^-$ (*lane 5*), S254F (*lane 6*), and S254F/*slr*0399$^-$ (*lane 7*) strains.** One primer located upstream and the other downstream of *slr*0399 have been used for the PCR amplification. Estimated sizes of the PCR products in kb are indicated on the *right*. *Lane 1*, 1-kb ladder.

D2R8 mutant has an apparently mobile Q_A resulting in inhibition of PS II electron transport by artificial quinones, most prominently duroquinone (DQ) (the I_{50} for inhibition of oxygen evolution in this mutant is 2 μM). Moreover, in the absence of artificial quinones, induction of variable fluorescence in D2R8 is very slow and the Q_A^-/donor side charge recombination kinetics have become slower (12). For this reason, the Q_A properties of the D2R8/*slr*0399$^-$ strain were characterized. As indicated in Fig. 3, in the D2R8/*slr*0399$^-$ strain the I_{50} value for the inhibition of oxygen evolution by DQ was about 25 μM, which is an order of magnitude higher than that for the D2R8 mutant. However, DQ is still a more potent inhibitor in the D2R8/*slr*0399$^-$ strain than in wild type. Other quinones (2,5-dichloro-*p*-benzoquinone, 2,5-dimethyl-*p*-benzoquinone) that inhibited electron transfer in the D2R8 mutant (12) had little effect on electron transfer in the D2R8/*slr*0399$^-$ strain (data not shown). Moreover, the D2R8/*slr*0399$^-$ strain displayed reasonably normal fluorescence induction kinetics (data not shown) and the yield of variable fluorescence (Table III) had increased significantly compared with D2R8. In addition, the rates of the Q_A^- oxidation by Q_B (in the absence of DCMU) and by charge recombination with the PS II donor side (in the presence of DCMU) in the D2R8/*slr*0399$^-$ strain were similar to those in the wild type (Table IV).

This reversal toward wild type properties upon inactivation of *slr*0399 was not specific for D2R8. In the S254F mutant fluorescence and other PS II properties were altered as well, although not as drastically as in D2R8, and were restored to essentially wild type characteristics upon inactivation of *slr*0399$^-$ (Tables III and IV).

Prenylquinone Analysis—The results presented above are indicative of an alteration of Q_A function in D2 mutants, but not in wild type, upon inactivation of *slr*0399. One possibility to lead to this phenotype is that the *slr*0399 gene product influences the quinone composition and/or the amount of quinone in the membranes of the organism. To determine whether this may be the case, all prenyllipids, including prenylquinones, were isolated from the D2R8, D2R8/*slr*0399$^-$, wild type, and *slr*0399$^-$ strains and separated by reverse-phase HPLC. HPLC separation of prenyllipids from the wild type and the *slr*0399$^-$ strain is presented in Fig. 4, indicating no major differences between these two strains, although the level of phylloquinone had been somewhat decreased in the mutant. The prenylquinone composition of the other two strains was also similar (data not shown). Only PQ and phylloquinone (vitamin K_1) were found as prenylquinones in *Synechocystis* sp. PCC 6803, similar to what has been reported before in other cyanobacterial species (26, 27). No new prenylquinone species were detected upon deletion of *slr*0399, indicating that the mechanism of the restoration of PS II function in pseudorevertants is not due to accommodation of a quinone different than PQ at the altered Q_A site.

Possible Functions of Slr0399—The results above indicate that Slr0399 is not involved with quinone synthesis, but a role of this protein as a chaperone in plastoquinone insertion into nascent photosystem II seems certainly a viable hypothesis.

TABLE III

Functional effects of the slr0399 deletion on photoautotrophic growth rates, PS II electron transport rates, the amount of chlorophyll per DCMU binding site, and the DCMU affinity in the wild type and two psbDI mutant strains of Synechocystis *sp. PCC 6803 with intact or inactivated slr0399*

Strain	Photoautotrophic doubling time	Oxygen evolution	Chl/DCMU binding site ratio	Ch1/DCMU binding site ratio	$K_{D(DCMU)}$	$(F_m - F_o)/F_m$
	h	*μmol O_2/(mg Chl) h*		*% of wild type*	*nM*	
D2R8	∞	100	3700 ± 600	15	66 ± 11	0.024 ± 0.007
D2R8R3	16	190	1060 ± 120	52	22 ± 2	0.36 ± 0.01
D2R8/*slr*0399$^-$	18	210	1200 ± 290	46	25 ± 2	0.37 ± 0.02
S254F	∞	120	3090 ± 110	18	31 ± 6	0.08 ± 0.008
S254F/*slr*0399$^-$	21	250	1110 ± 110	50	19 ± 0.5	0.22 ± 0.03
Wild type	12	320	550 ± 30	100	17 ± 1	0.49 ± 0.01
*slr*0399$^-$	13	315	540 ± 120	102	19 ± 1	0.49 ± 0.02

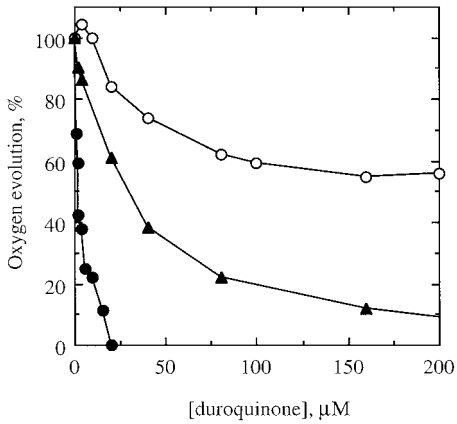

FIG. 3. **Inhibition of steady-state oxygen evolution in continuous light by DQ in the D2R8 (●), D2R8/*slr*0399$^-$ (▲), and wild type (○) strains.** Oxygen evolution was measured at saturating light intensity and in the presence of 0.5 mM $K_3[Fe(CN)_6]$ (which does not penetrate cells) to keep DQ oxidized.

Slr0399 consists of 326 amino acids and is similar to the putative protein of unknown function Ycf39 that is encoded in the cyanelle genome of *Cyanophora paradoxa*[2] (53% identity, 73% similarity) and in the chloroplast genomes of non-green algae including *Ochrosphaera neapolitana*[3] (65% similarity, 43% identity), *Odontella sinensis*[4] (69% similarity, 45% identity) (38), *Porphyra purpurea*[5] (72% similarity, 50% identity) (29), and *Cyanidium caldarium*[6] (55% similarity, 35% identity). It is also similar to the predicted translation product of an *A. thaliana* nuclear gene (76% similarity and 58% identity) located on chromosome 4, BAC clone F23E12.[7]

An alignment of Slr0399 and its homologues from non-green algae and *Arabidopsis* is provided in Fig. 5. Regions of high similarity between Slr0399 and Ycf39 proteins are scattered throughout the protein. The only clearly identifiable functional domain in Slr0399 is a conserved NAD(P)H-binding motif near the N terminus of the protein (Fig. 5). This putative nucleotide-binding domain contains a β-α-β-α-β motif, which is present in all classical dinucleotide binding proteins (for review, see Ref. 30), and which interacts with the adenosine pyrophosphoryl moiety of the cofactor (NAD, NADP, or possibly FAD). Residues 3–32 of Slr0399 constitute the fingerprint region (β-α-β), derived from known structures of dinucleotide-binding enzymes (31, 32). This region contains a glycine-rich phosphate-binding consensus sequence G(*X*)*X*G*XX*G and six conserved hydrophobic residues at characteristic locations (marked with *asterisks* in Fig. 5). The absence of a conserved Asp or Glu at the carboxyl end of the β-α-β motif and the presence a conserved Arg residue instead (Fig. 5) suggest that Slr0399 binds NADPH rather than NADH (33, 34). Moreover, a fingerprint motif present in FAD-binding enzymes (35) is not obvious in Slr0399, arguing against the possibility that FAD would serve as a cofactor in this protein.

The position of this highly conserved region with the NAD(P)H-binding motif so close to the N-terminal end of the protein sequence suggests that Slr0399/Ycf39 are not processed in the cyanobacterium or in eukaryotes when this protein is chloroplast-encoded. Therefore, Slr0399 is expected to remain in the cytoplasm in *Synechocystis* sp. PCC 6803 (and Ycf39 is expected to be located in the chloroplast stroma) and to not be translocated into the lumen. The presence of a putative chloroplast targeting leader sequence in the *A. thaliana* nuclear-encoded Ycf39 protein (PSORT software; Refs. 36 and 37) is in agreement with targeting into the chloroplast stroma (data not shown).

Slr0399 and its Ycf39 homologues from eukaryotes are mostly hydrophilic. Slr0399 has a single hydrophobic domain that is long enough to span the thylakoid membrane (Tyr-139 to Leu-160),[8] but because in Ycf39 homologues this region sometimes carries charges it is unlikely that this domain is an actual membrane-spanning region. Also, this region is unlikely to form an α-helix,[8] and therefore, this protein is likely to not span the membrane.

Slr0399 Effect on Thermotolerance—As indicated earlier, a possible explanation of the data presented in this paper is that Slr0399 is a chaperone-like protein that aids in, but is not essential for, quinone insertion and protein folding around Q_A in photosystem II. Lack of chaperone-like proteins sometimes leads to a thermosensitive phenotype (41). To determine the temperature sensitivity in relation to the presence of Slr0399, the wild type and *slr*0399$^-$ *Synechocystis* sp. PCC 6803 strains were first grown at 30 °C at 50 μmol photons m^{-2} s^{-1} light intensity and were then diluted and transferred to 39 °C at the same light intensity. This temperature is close to the temperature maximum (40–44 °C) for photoautotrophic growth of *Synechocystis* sp. PCC 6803 wild type (42). The photoautotrophic growth rate of both strains immediately after transfer to

[2] V. L. Stirewalt, C. B. Michalowski, W. Löffelhardt, H. J. Bohnert, and D. A. Bryant, GenBank™ accession no. U30821.

[3] V. A. R. Huss, A. C. Tietze, and C. Julius, C., GenBank™ accession no. X99077.

[4] GenBank™ accession no. Z67753.

[5] GenBank™ accession no. U38804.

[6] G. Gloeckner, A. Rosenthal, and K. Valentin, GenBank™ accession no. AF022186.

[7] M. Bevan, H. Hilbert, M. Braun, E. Holzer, A. Brandt, A. Duesterhoeft, J. Hoheisel, T. Jesse, L. Heijnen, P. Vos, H. W. Mewes, K. F. X. Mayer, and C. Schueller, GenBank™ accession no. AL022604.

[8] Software used for the protein secondary structure analysis was as follows: hydropathy analysis using algorithm of Kyte and Doolittle (38), DAS (39), TMpred, and SOSUI (40).

TABLE IV

Kinetics of Q_A^- oxidation in the presence and absence of DCMU in the D2R8 and S254F mutants and the wild type strain with intact or inactivated slr0399

Chlorophyll *a* fluorescence decay traces were deconvoluted assuming the presence of two exponential components; if a one-component deconvolution provided an equally good fit, the second component was omitted. A_1 and A_2 represent the amplitudes of the two phases (normalized to give a sum of 100); $(t_{1/2})_1$ and $(t_{1/2})_2$ are the corresponding decay half-times. Data were reproducible within about 15%. Note that the variable fluorescence intensity of D2R8 is very small, and in this strain the error therefore is larger (up to about 25%).

Strain	−DCMU				+DCMU			
	A_1	$(t_{1/2})_1$	A_2	$(t_{1/2})_2$	A_1	$(t_{1/2})_1$	A_2	$(t_{1/2})_2$
		μs		*ms*		*s*		*s*
D2R8	100	830			100	1.59		
D2R8/*slr*0399$^-$	78	510	22	3.2	40	0.18	60	0.92
S254F	83	430	17	4.2	54	0.35	46	2.2
S254F/*slr*0399$^-$	53	230	47	1.4	56	0.21	44	0.99
Wild type	65	340	35	2.0	48	0.24	52	0.98
*slr*0399$^-$	64	300	36	1.9	48	0.21	52	0.74

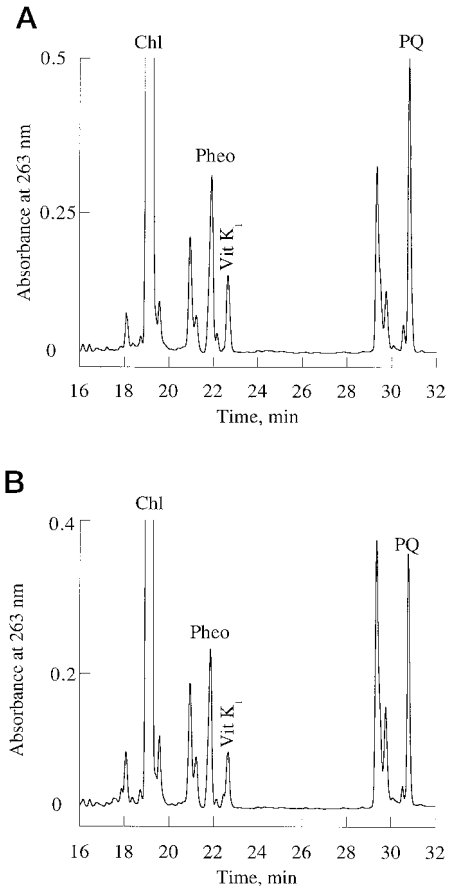

FIG. 4. **HPLC analysis of prenyllipids isolated from membranes of the wild type (*A*) and *slr*0399$^-$ (*B*) strains of *Synechocystis* sp.** PCC 6803. The peaks identified are chlorophyll *a* (*Chl*), pheophytin (*Pheo*), phylloquinone (*Vit K_1*), and plastoquinone (*PQ*). Only oxidized forms of prenylquinones are present since membranes were treated with 0.5 mM $K_3[Fe(CN)_6]$ prior to extraction.

39 °C was very similar and almost twice as fast as observed at 30 °C. However, after about 5 cell divisions, the *slr*0399$^-$ culture abruptly stopped dividing and eventually died, whereas the control strain continued growing at a rapid rate (Fig. 6*A*). The cessation of growth of the *slr*0399$^-$ strain was related to the number of cell divisions rather than to the length of 39 °C exposure because at limiting light intensity (9 μmol photons m^{-2} s^{-1}) where the growth rate of the wild type and *slr*0399$^-$ strains had decreased by a factor of 3, the cell division in the *slr*0399$^-$ strain stopped after a 3-fold longer heat exposure (corresponding again to 5 cell divisions) (data not shown). The inability of the *slr*0399$^-$ strain to sustain photoautotrophic growth at 39 °C was not due solely to inactivation of the PS II complex, since addition of glucose did not alleviate the cessation of growth (Fig. 6*B*). Furthermore, in the presence of glucose *slr*0399$^-$ cells stopped dividing even earlier, after about 3 to 4 cell divisions at 39 °C (Fig. 6*B*). This indicates that Slr0399 appears to serve a chaperone function involving complexes other than PS II as well. Indeed, quinone-binding complexes are common in both photosynthetic and respiratory electron transfer, and therefore a role of Slr0399 that goes beyond PS II is not unexpected.

DISCUSSION

A novel technique of functional complementation with size-separated restriction fragment pools (3) used in this work for the localization of a pseudoreversion has become feasible with the availability of the entire *Synechocystis* sp. PCC 6803 genome sequence (4) and, hence, its genomic restriction map. This method exploits the ability of this naturally transformable cyanobacterium to integrate exogenous DNA into its genome by homologous recombination (1, 2). Application of this procedure in *Synechocystis* sp. PCC 6803 is a powerful and elegant approach toward mapping the sites of secondary mutations in pseudorevertants, as well as of spontaneous mutations and mutations introduced via random mutagenesis. A main factor determining the suitability of this approach is whether a strong selection for screening of complemented transformants is available. In the case of the work described here, this strong selection was provided by restoration of photoautotrophic growth.

In all D2R8 pseudorevertants that have been mapped, photoautotrophic growth was restored due to secondary mutations in *slr*0399, an open reading frame on the *Synechocystis* sp. PCC 6803 chromosome. Targeted deletion of the entire 3′-terminal half of *slr*0399 also restored photoautotrophic growth in D2R8. The D2R8 pseudorevertants and the D2R8/*slr*0399$^-$ mutant showed increased PS II levels as compared with D2R8 (Table III), and the functional characteristics of Q_A were restored to close to wild type values (Table IV and Fig. 3). Interestingly, inactivation of *slr*0399 also restored photoautotrophic growth and PS II properties in some other obligate photoheterotrophic *psb*DI mutants that had alterations around the Q_A site and that retained some oxygen evolution. However, other PS II mutants could not be restored by inactivation of *slr*0399 (Table II), suggesting a specific interaction between Slr0399 and the Q_A site of PS II.

The *Synechocystis* sp. PCC 6803 open reading frame *slr*0399 is expected to encode a 36-kDa protein, which shows high similarity with hypothetical protein Ycf39 (Fig. 5). It is noteworthy that the *ycf*39 gene, present in chloroplast genomes in non-green algae, was transferred to the nuclear genome later in evolution of plants. This protein contains a putative

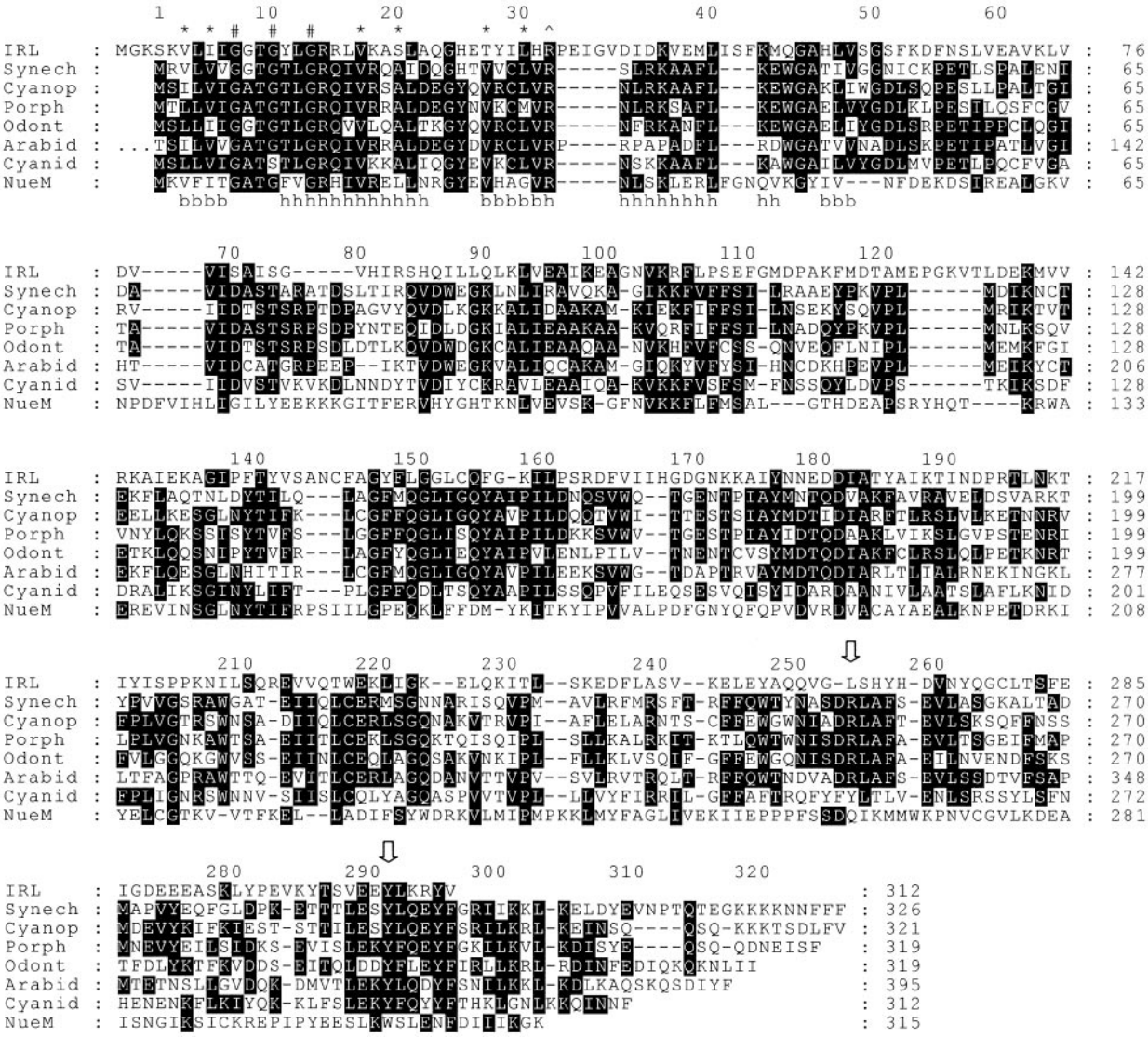

FIG. 5. **Alignment of Slr0399 and its homologues from non-green algae and *Arabidopsis*.** Figure shows alignment of the deduced amino acid sequence of Slr0399 from *Synechocystis* sp. PCC 6803 (*Synech*) with Ycf39 sequences from *C. paradoxa* (footnote 2; *Cyanop*), *P. purpurea* (footnote 5; *Porph*), *O. sinensis* (footnote 4; *Odont*), *A. thaliana* (footnote 7; *Arabid*), and *C. caldarium* (footnote 6; *Cyanid*), and with a putative NADH:ubiquinone oxidoreductase subunit from *A. aeolicus* (footnote 9; *NueM*) and a (+)-pinoresinol/(+)-lariciresinol reductase (GenBank™ accession no. U81158), an enzyme from the isoflavone reductase-like protein family (*IRL*). The leader peptide in the Ycf39 sequence from *A. thaliana* is not shown. Alignment was produced using the PILEUP program of the University of Wisconsin Computer Group with the following settings: gap introduction penalty 7, and gap extension penalty 2. Amino acid residues that are identical in at least 3 out of the 6 Ycf39 homologues (Slr0399 included) have been *boxed*. Amino acid residues in the IRL and NueM protein sequences that are identical to residues conserved in at least 3 out of 6 Ycf39 homologues have also been *boxed*. Residues that constitute the NAD(P)H binding motif have been marked by # (invariant glycine residues), ★ (hydrophobic residues), and ∧ (a conserved Arg residue). Computer-predicted[8] secondary structure of this domain has been indicated under the alignment (b = β-sheet, h = α-helix). Residues mutated in pseudorevertants D2R8R1 (Y291C) and D2R8R3 (R254H) have been marked by *arrows*. Numbering above the sequences is according to Slr0399.

NAD(P)H-binding motif and has an overall similarity with two groups of proteins. One group is the family of isoflavone reductase-like (IRL) proteins that are present in different plants species (43–48). Typically, Slr0399 is 25% identical and 40% similar to IRL proteins from plants (Fig. 5); the level of similarity to other groups of reductases is much lower (data not shown). Slr0399 also shares similarity (about 20% identity and 40% similarity) with NueM[9] from the hyperthermophilic bacterium *Aquifex aeolicus* that has been identified as a subunit of a NADH:ubiquinone oxidoreductase on the basis of its sequence similarity with the 39-kDa subunit of the bovine mitochondrial complex I (49).

The IRL proteins have been grouped in a family solely on the basis of their high sequence similarity with isoflavone reductases, which catalyze the reduction of α,β-unsaturated ketones and which are involved in biosynthesis of isoflavonoid phytoalexins in legumes in response to fungal infection (50–52). However, IRL proteins have been identified in plants that do not synthesize isoflavonoid phytoalexins in response to pathogen attack (53), and therefore IRLs are likely to have broader functions. IRLs have been implied to function in response to oxidative stress in *Arabidopsis* (44), prolonged sulfur starvation in maize (45), and to UV radiation in harvested grapefruit (48). It has been suggested that all isoflavone reductase-like proteins are oxidoreductases utilizing NAD(P)H as a cofactor, which have various substrates that may or may not be related structurally to flavonoids (43, 44).

The similarity of Slr0399 with NueM[9] from *Aquifex aeolicus*

[9] GenBank™ accession no. AE000675.

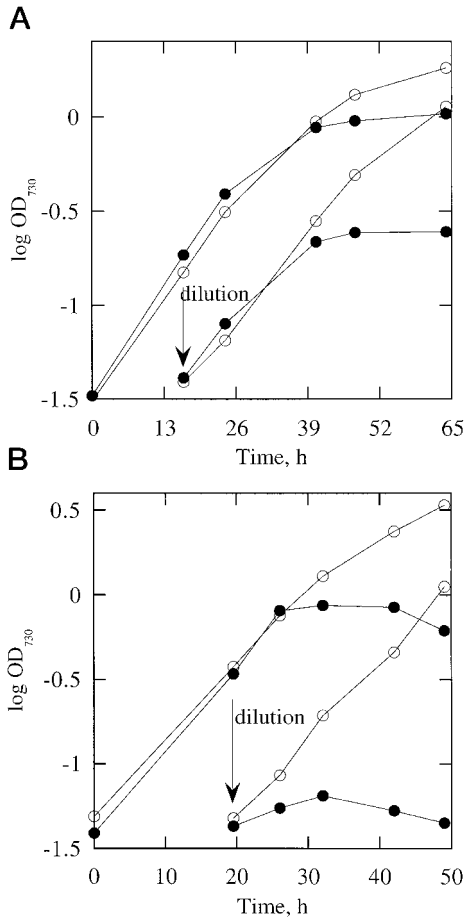

FIG. 6. **Photoautotrophic (*A*) and photomixotrophic (*B*) growth of the wild type (○) and *slr*0399$^-$ (●) strains of *Synechocystis* sp.** PCC 6803 at 39 °C. Strains had been grown under photoautotrophic conditions at 30 °C, and at time 0 the cultures were diluted and transferred to 39 °C.

and with 39/40-kDa subunit of the NADH:ubiquinone oxidoreductase complex I of fungi and mammals (54, 55) may be important, as these proteins may interact with quinones. The peripheral 39/40-kDa subunit of complex I in eukaryotes has no homologue in the "minimal" 14-subunit bacterial respiratory NADH dehydrogenase. The precise function of this subunit remains unknown.

The fact that inactivation of *slr*0399 can complement different mutations in the Q_A- binding region of the D2 protein and does not complement mutations in several other PS II subunits suggested to us that Slr0399 may be involved with PQ metabolism or biosynthesis, PQ incorporation into PS II centers, Q_A stabilization, or with regulation of the PQ pool size or the redox state of the thylakoid membrane. The hypothesis of Slr0399 involvement in quinone biosynthesis or metabolism (for example, leading to accumulation of quinones that can bind tightly to the altered Q_A site in the D2R8 mutant and that are functional at this site) is countered by our observation that no significant qualitative or quantitative changes in prenylquinone composition occur in *Synechocystis* sp. PCC 6803 strains upon deletion of *slr*0399. Therefore, we do not favor a role of Slr0399 in prenylquinone biosynthesis or metabolism.

As indicated in Fig. 4, in the *slr*0399$^-$ mutant the amount of PQ remained rather constant, even though the amount of phylloquinone decreased somewhat. Most, if not all, phylloquinone in thylakoid membranes in cyanobacteria is believed to be associated with PS I centers in a stochiometry of 2 quinone molecules per PS I complex (56, 57). This suggests that the amount of PS I-associated phylloquinone is decreased in the *slr*0399$^-$ strain; however, based on the comparison of OD_{730} and chlorophyll amounts, there is no evidence for a decrease in the amount of PS I per cell in the *slr*0399$^-$ mutant (data not shown). A possible explanation is that Slr0399 is involved in insertion of one of the phylloquinones into PS I as well, but the lack of phenotypic consequences of *slr*0399 deletion excludes the possibility that this phylloquinone is functionally critical.

Another possible explanation for the improvement of PS II function in D2R8 and S254F by inactivation of *slr*0399 would be that deletion of *slr*0399 changes the redox state of the PQ pool, leading to stabilization of Q_A function in the mutants. However, determination of fluorescence induction curves in the presence and absence of PS I did not show significant changes upon inactivation of *slr*0399 (data not shown), suggesting that the redox state of the system is not significantly altered by Slr0399. Moreover, there is no evidence that Slr0399 is a structural component of PS II, thereby making it unlikely that Slr0399 continuously interacts with Q_A, either directly or indirectly.

The working hypothesis that we favor is that Slr0399 is a chaperone-like protein that is involved in (but not crucial for) Q_A insertion into PS II centers, possibly delivering the quinone to the nascent reaction center in a particular redox state. In wild type, this leads to stable reaction centers, but in strains with mutations in the Q_A niche the folded D2 protein does not lock Q_A in place. However, in such strains Q_A apparently can be locked in place in the majority of centers if Slr0399 has been truncated or altered. Whether this modified protein, another protein, or even no protein assists in Q_A assembly in this mutant system is beyond the scope of this study. This working hypothesis of Slr0399 as a chaperone-type protein helping in insertion of cofactors is supported by the observation that the slr0399 inactivation mutant has a temperature-sensitive phenotype (Fig. 6). The fact that this phenotype persists even in the presence of glucose (when no PS II activity is needed for growth) suggests that the function of Slr0399 is not limited to assembly of PS II, but may involve other quinone-binding complexes such as PS I and the NADH:ubiquinone oxidoreductase complex I.

In the *Synechocystis* sp. PCC 6803 genome, there are two other open reading frames encoding putative proteins that are similar to the N-terminal 150–200 amino acids of Slr0399: *sll*1218 (31% identity and 52% similarity of the corresponding polypeptide with Slr0399) and *slr*0317 (23% identity and 42% similarity). The calculated molecular masses of Sll1218 and Sll0317 are 24 and 32 kDa, respectively. Both proteins contain a NAD(P)H-binding domain near the N terminus, suggesting that these two hypothetical proteins are dehydrogenases or reductases, but they may differ from Slr0399 in their specific function.

Acknowledgments—We are grateful to Drs. Hadar Kless, Bruce Diner, and Achim Trebst for helpful discussions and suggestions. We thank Dr. Dan Brune for help and advice on analysis of prenylquinones and Dr. Arcady Mushegian for his help with computer analysis of protein sequences.

REFERENCES

1. Grigorieva, G., and Shestakov, S. (1982) *FEMS Microbiol. Lett.* **13,** 367–370
2. Williams, J. G. K. (1988) *Methods Enzymol.* **167,** 766–778
3. Vermaas, W. F. J. (1998) *Methods Enzymol.* **297,** 293–310
4. Kaneko, T., Sato, S., Kotani, H., Tanaka, A., Asamizu, E., Nakamura, Y., Miyajima, N., Hirosawa, M., Sugiura, M., Sasamoto, S., Kimura, T., Hosouchi, T., Matsuno, A., Muraki, A., Nakazaki, N., Naruo, K., Okumura, S., Shimpo, S., Takeuchi, C., Wada, T., Watanabe, A., Yamada, M., Yasuda, M., and Tabata, S. (1996) *DNA Res.* **3,** 185–209
5. Nixon, P. J., Rögner, M., and Diner, B. A. (1991) *Plant Cell* **3,** 383–395
6. Kless, H., and Vermaas, W. (1995) *J. Biol. Chem.* **270,** 16536–16541
7. Chen, Z. X., Yu, W. Z., Lee, J. H., Diao, R., and Spreitzer, R. J. (1991) *Biochemistry* **30,** 8846–8850
8. Thow, G., Zhu, G., and Spreitzer, R. J. (1994) *Biochemistry* **33,** 5109–5114

9. Yu, J., and McIntosh, L. (1998) *Methods Enzymol.* **297,** 18–27
10. Girard-Bascou, J., Pierre, Y., and Drapier, D. (1992) *Curr. Genet.* **22,** 47–52
11. Wu, H. Y., and Kuchka, M. R. (1995) *Curr. Genet.* **27,** 263–269
12. Ermakova-Gerdes, S., and Vermaas, W. F. J. (1998) *Biochemistry* **37,** 11569–11578
13. Fink, A. L. (1999) *Physiol. Rev.* **79,** 425–449
14. Ellis, R. J., and Hartl, F. U. (1999) *Curr. Opin. Struct. Biol.* **9,** 102–110
15. Rippka, R., Deruelles, J., Waterbury, J. B., Herdman, M., and Stanier, R. Y. (1979) *J. Gen. Microbiol.* **111,** 1–61
16. Vermaas, W. F. J., Charité, J., and Shen, G. (1990) *Z. Naturforsch.* **45c,** 359–365
17. Kless, H., and Vermaas, W. (1995) *J. Mol. Biol.* **246,** 120–131
18. He, Q., and Vermaas, W. (1998) *Proc. Natl. Acad. Sci. U. S. A.* **95,** 5830–5835
19. Kalén, A., Soderberg, M., Elmberger, P. G., and Dallner, G. (1990) *Lipids* **25,** 93–99
20. Koivuniemi, A., Swiezewska, E., Aro, E.-M., Styring, S., and Andersson, B. (1993) *FEBS Lett.* **327,** 343–346
21. Cramer, W. A., and Knaff, D. B. (1991) *Energy Transduction in Biological Membranes: A Textbook of Bioenergetics,* Springer-Verlag, New York
22. Morton, R. A. (1965) in *Biochemistry of Quinones* (Morton, R. A., ed) pp. 23–66, Academic Press, London
23. Ermakova-Gerdes, S., Shestakov, S., and Vermaas, W. (1996) *Plant Mol. Biol.* **30,** 243–254
24. Kless, H., Oren-Shamir, M., McIntosh, L., and Edelman, M. (1994) *Biochemistry* **33,** 10501–10507
25. Haag, E., Eaton-Rye, J. J., Renger, G., and Vermaas, W. F. J. (1993) *Biochemistry* **32,** 4444–4454
26. Carr, N. G., Exell, G., Flynn, V., Hallaway, M., and Talukdar, S. (1967) *Arch. Biochem. Biophys.* **120,** 503–507
27. Katoh, S., and Satoh, K. (1988) *Methods Enzymol.* **167,** 269–271
28. Kowallik, K. V., Stoebe, B., Schaffran, I., Kroth-Pancic, P., and Freier, U. (1995) *Plant Mol. Biol. Rep.* **13,** 336–342
29. Reith, M. E., and Munholland, J. (1995) *Plant Mol. Biol. Rep.* **13,** 333–335
30. Bellamacina, C. R. (1996) *FASEB J.* **10,** 1257–1269
31. Rossmann, M. G., Moras, D., and Olsen, K. W. (1974) *Nature* **250,** 194–199
32. Wierenga, R. K., Terpstra, P., and Hol, W. G. J. (1986) *J. Mol. Biol.* **187,** 101–107
33. Wierenga, R. K., DeMaeyer, M. C. H., and Hol, W. G. J. (1985) *Biochemistry* **24,** 1346–1357
34. Brandén, C., and Tooze, J. (1991) *Introduction to Protein Structure,* Garland Publishing, Inc., New York
35. Eggink, G., Engel, H., Vriend, G., Terpstra, P., and Witholt, B. (1990) *J. Mol. Biol.* **212,** 135–142
36. von Heijne, G., Steppuhn, J., Herrmann, R. G. (1989) *Eur. J. Biochem.* **180,** 535–545
37. Schnell, D. J. (1998) *Annu. Rev. Physiol. Plant Mol. Biol.* **49,** 97–126
38. Kyte, J., and Doolittle, R. F. (1982) *J. Mol. Biol.* **157,** 105–132
39. Cserzo, M., Wallin, E., Simon, I., von Heijne, G., and Elofsson, A. (1997) *Protein Eng.* **10,** 673–676
40. Hirokawa, T., Boon-Chieng, S., and Mitaku, S. (1998) *Bioinformatics* **14,** 378–379
41. Parsell, D. A., and Lindquist, S. (1993) *Annu. Rev. Genet.* **27,** 437–496
42. Lehel, C., Gombos, Z., Török, Z., and Vigh, L. (1993) *Plant Physiol. Biochem.* **31,** 81–88
43. Hibi, N., Higashiguchi, S., Hashimoto, T., and Yamada, Y. (1994) *Plant Cell* **6,** 723–735
44. Babiychuk, E., Kushnir, S., Belles-Boix, E., Van Montagu, M., and Inzé, D. (1995) *J. Biol. Chem.* **270,** 26224–26231
45. Petrucco, S., Bolchi, A., Foroni, C., Percudani, R., Rossi, G. L., and Ottonello, S. (1996) *Plant Cell* **8,** 69–80
46. Dinkova-Kostova, A. T., Gang, D. R., Davin, L. B., Bedgar, D. L., Chu, A., and Lewis, N. G. (1996) *J. Biol. Chem.* **271,** 29473–29482
47. van Eldik, G. J., Ruiter, R. K., Colla, P. H. W. N., van Herpen, M. M. A., Schrauwen, J. A. M., and Wullems, G. J. (1997) *Plant Mol. Biol.* **33,** 923–929
48. Lers, A., Burd, S., Lomaniec, E., Droby, S., and Chalutz, E. (1998) *Plant Mol. Biol.* **36,** 847–856
49. Deckert, G., Warren, P. V., Gaasterland, T., Young, W. G., Lenox, A. L., Graham, D. E., Overbeek, R., Snead, M. A., Keller, M., Aujay, M., Huber, R., Feldman, R. A., Short, J. M., Olson, G. J., and Swanson, R. V. (1998) *Nature* **392,** 353–358
50. Paiva, N. L., Edwards, R., Sun, Y., Hrazdina, G., and Dixon, R. A. (1991) *Plant Mol. Biol.* **17,** 653–667
51. Tiemann, K., Inzé, D., van Montagu, M., and Barz, W. (1991) *Eur. J. Biochem.* **200,** 751–757
52. Dixon, R. A., Harrison, M. J., and Paiva, N. L. (1995) *Physiol. Plant.* **93,** 385–392
53. Dixon, R. A., Dey, P. M., and Lamb, C. J. (1983) in *Advances in Enzymology and Related Areas in Molecular Biology* (Meister, A., ed) pp.1–83, John Wiley & Sons, New York
54. Röhlen, D. A., Hoffmann, J., van der Pas, J. C., Nehls, U., Preis, D., Sackman, U., and Weiss, H. (1991) *FEBS Lett.* **278,** 75–78
55. Fearnley, I. M., Finel, M., Skehel, J. M., and Walker, J. E. (1991) *Biochem. J.* **278,** 821–829
56. Takahashi, Y., Hirota, K., and Katoh, S. (1985) *Photosynth. Res.* **6,** 183–192
57. Schroeder, H.-U., and Lockau, W. (1986) *FEBS Lett.* **199,** 23–27

Inactivation of the Open Reading Frame *slr*0399 in *Synechocystis* sp. PCC 6803 Functionally Complements Mutations near the Q_A Niche of Photosystem II: A POSSIBLE ROLE OF Slr0399 AS A CHAPERONE FOR QUINONE BINDING

Svetlana Ermakova-Gerdes and Wim Vermaas

J. Biol. Chem. 1999, 274:30540-30549.
doi: 10.1074/jbc.274.43.30540